Correlation of Serum and Urine Enzyme Activity in Patients with Acute Myocardial Infarction

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A correlation was made between the urinary excretion of GOT, GPT, LDH, HBD, and acid and alkaline phosphatase and their serum activity in 18 suspected cases of myocardial infarction. The diagnoses were made by a cardiologist with knowledge of the enzyme assays. The study included 2 cases of nontransmural infarction; 5 cases that had not sustained an infarction served as controls. Elevations of serum GOT, LDH, and HBD were found in 10 of 11 cases of transmural infarction, GPT and acid phosphatase in 5, and alkaline phosphatase in 7. Elevations in serum acid phosphatase were also found in the control group, and perhaps were related to the anticoagulation therapy. Urinary GOT and GPT activity was variable. Urinary LDH and HBD were elevated in 6 of the transmural infarction cases, usually when serum LDH and HBD activity was decreasing. Urinary acid phosphatase activity was essentially normal; but urinary alkaline phosphatase increased in 10 of the transmural infarction cases, and the average value remained high for more than 5 weeks. The only index that was elevated in all cases of acute transmural infarction was L-phenylalanine inhibition of urinary alkaline phosphatase activity.

Since the discovery of elevations of serum activities of glutamic oxaloacetic transaminase (GOT) by LaDue et al. (1) and lactate dehydrogenase (LDH) by Wróblewski and LaDue (2) in patients with recent myocardial infarctions, much interest has been centered in these and other serum enzymes in the differential diagnosis of heart disease. Although a number of enzymes present in serum also are found in the urine, there have been virtually no reports on urinary enzymes in myocardial infarction. Kalmansohn and Kalmansohn (3) reported that urinary GOT activity was elevated in some patients with myocardial infarction, and Dubach and Rediger (4) found a slight elevation of urinary LDH in 1 patient on the sixth postinfarction day.

This report presents the results of a study of the pattern of urinary

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enzyme activity following myocardial infarction, correlated with serum activity of the same enzymes. The enzymes studied were alkaline and acid phosphatase, LDH, α-hydroxybutyric dehydrogenase (HBD), GOT, and glutamic pyruvic transaminase (GPT).

Material and Methods

Subjects

Eighteen male patients were studied, all initially thought to have sustained an acute myocardial infarction less than 48 hr. prior to the study. The final diagnosis was made by one of us (D. F.) independently of the enzyme findings. On the basis of clinical and electrocardiographic evidence, the patients were divided into 3 groups: (1) those with acute transmural myocardial infarction (11 patients); (2) those with non-transmural infarction (2 patients); and (3) the remaining 5 patients, 2 of whom showed evidence of left ventricular hypertrophy, 1 of anterior wall ischemia, and 2 had normal electrocardiographic tracings. These last 5 patients comprised the control group. All patients were treated initially with heparin and then with Coumadin.

Blood and Urine Collection

Initial blood and urine specimens were obtained shortly after hospital admission, and urine samples were collected continuously for 14 days. Blood was drawn daily for the first 3 postinfarction days and on the fifth, seventh, tenth, and fourteenth days. Thereafter, 24-hr. urine specimens and a blood specimen were obtained weekly. This routine was continued until discharge, usually in the fifth week. The weight and height of the patients were measured as soon as each patient could be moved.

Procedure

Thymol was added to the urine as a preservative and the specimens kept as cool as possible during collection. Urine samples collected during the day were refrigerated; the next morning they were added to the overnight samples. A portion of the 24-hr. collection was centrifuged and the sediment examined. The centrifuged supernatant was divided into 3 portions: (1) one portion was used directly for the assay of acid phosphatase and creatinine; (2) a 30-ml aliquot was dialyzed for 4 hr, against 4 L. of distilled water at 4°, then diluted with distilled water to 35 ml. and used immediately for the assay of alkaline phosphatase, LDH, HBD, GOT, and GPT; and (3) a 300-ml. aliquot was exhaustively dialyzed against tap water, then against three changes of 12 L. each of distilled water for 12 hr. each. The dialysate was lyophilized and the
residue weighed to give the total nondialyzable solids. The average excretion of the nondialyzable solids was the same in all groups, about 0.40 mg./kg./hr.

**Assays**

All assays were based on standard procedures and carried out at 37°. A summary of the assay conditions is given in Table 1.

The phosphatase assays were based on the method of Bessey et al. (5) using p-nitrophenyl phosphate (NPP) as substrate. Acid phosphatase was assayed in acetate buffer of pH 4.75 and alkaline phosphatase in 2-amino-2-methyl-1,3-propanediol (AMP) buffer of pH 9.75. The AMP buffer was prepared by mixing 0.2 M AMP and 0.2 M HCl to obtain the desired pH. Mg++ was included in the assay for alkaline phosphatase. Both serum and urine were assayed by the same system. Undialyzed urine is best for the assay of acid phosphatase, but dialyzed urine is needed for the assay of alkaline phosphatase (6). The inhibition of acid phosphatase by Cu++ (1 mM) and by tartrate (2 mM) was measured; this tartrate concentration gives approximately 70% inhibition in normal urine. The inhibition of alkaline phosphatase by 5 mM L-phenylalanine was also determined.

GOT, GPT, and LDH were determined by the method of Henry et al. (7). For HBD, the method of Elliott and Wilkinson (8) was employed; except for the substrate, this method is essentially the same as that of Henry et al. (7) for LDH. Both methods involve the oxidation of nicotinamide adenine dinucleotide (NADH) in the presence of the proper substrate; the decrease in absorbance (∆A) was followed on a Beckman DB spectrophotometer using a Sargent SLR recorder. Amador et al. (9) did not find this method for LDH to be linear, but our results are in agreement with those of Henry et al. (7) in that the reaction is linear with time and sample size. An example of the latter is shown in Fig. 1 for both serum and urine. Although there is disagreement in the literature as to the need for dialysis in urinary LDH measurement, our results show higher values for dialyzed urine. The assay results of 17 normal and pathologic samples before and after dialysis are shown in Fig. 2. The small area in the lower left corner represents the normal range. The dialyzed samples were more active by a mean value of 4.45 ± 7.75 nmole/min./kg./hr. (p < 0.05).

All values for serum assays are reported in international units, micromoles per minute per liter. Urine values are reported as nanomoles per minute of assay per kilogram of body weight per hour of urine collection (6).

Creatinine clearances were determined daily on the 24-hr. urine col-
Table 1. Enzyme Assay Conditions

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Buffer Type</th>
<th>pH</th>
<th>Concentration (μM)</th>
<th>Substrate Type</th>
<th>Concentration (mM)</th>
<th>Cofactor Type</th>
<th>Concentration (mM)</th>
<th>Sample (ml.)</th>
<th>Incubation (°C)</th>
</tr>
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<tbody>
<tr>
<td>AcP†</td>
<td>Acetate</td>
<td>4.85</td>
<td>0.06</td>
<td>NPP</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>0.2</td>
<td>0.04 5 60</td>
</tr>
<tr>
<td>AIP‡</td>
<td>AMP</td>
<td>9.85</td>
<td>0.036</td>
<td>NPP</td>
<td>3</td>
<td>MgCl₂</td>
<td>1</td>
<td>0.04</td>
<td>1.71 5 60</td>
</tr>
<tr>
<td>LDH</td>
<td>Phosphate</td>
<td>7.4</td>
<td>0.1 M</td>
<td>Pyruvate</td>
<td>0.6</td>
<td>NADHₖ</td>
<td>0.187</td>
<td>0.1</td>
<td>0.42 3 5</td>
</tr>
<tr>
<td>HBD</td>
<td>Phosphate</td>
<td>7.4</td>
<td>0.1 M</td>
<td>α-Ketobutyrate</td>
<td>3.33</td>
<td>NADHₖ</td>
<td>0.093</td>
<td>0.1</td>
<td>0.42 3 5</td>
</tr>
<tr>
<td>GOT</td>
<td>Phosphate</td>
<td>7.4</td>
<td>0.1 M</td>
<td>L-Aspartate</td>
<td>125</td>
<td>MDHᵦ</td>
<td>1000 U.</td>
<td>0.2</td>
<td>0.17 3 8</td>
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<tr>
<td>GPT</td>
<td>Phosphate</td>
<td>7.4</td>
<td>0.1 M</td>
<td>α-Ketoglutarate</td>
<td>6.7</td>
<td>NADHₖ</td>
<td>0.187</td>
<td>0.2</td>
<td>0.17 3 8</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>dl-Alanine</td>
<td>333</td>
<td>LDH</td>
<td>800 U.</td>
<td>0.2</td>
<td>0.17 3 8</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>α-Ketoglutarate</td>
<td>6.7</td>
<td>NADHₖ</td>
<td>0.187</td>
<td>0.2</td>
<td>0.17 3 8</td>
</tr>
</tbody>
</table>

*All concentrations are those found in the assay mixture; the volumes of serum and urine are those most commonly used.
†Acid phosphatase.
‡Alkaline phosphatase.
ᵦMalic dehydrogenase.
lection. The procedure of Clark and Thompson (10) was used to measure urinary creatinine. Serum creatinine was determined after adsorption on Lloyd's reagent. When an occasional poor collection was revealed by a sudden drop in creatinine excretion, the urine sample was discarded. The creatinine clearances did not suggest renal impairment in any of the patients.

Results

Dehydrogenases and Transaminases

The average daily results for serum activity of 6 enzymes in 11 patients with transmural infarction are shown in Fig. 3. The results for LDH, HBD, GOT, and GPT were similar to those frequently reported. Serum GOT was maximal on the first postinfarction day and decreased rapidly thereafter. Serum GPT was less elevated but for a longer period than GOT, giving a secondary peak at about 5 days. Ten of the 11 patients with transmural infarction had elevations of serum GOT; only 6 showed increases in GPT activity. Maximum levels for LDH and HBD occurred somewhat later than those for transaminases. One patient with a transmural infarction had no elevation of serum LDH or HBD, and the 2 patients with nontransmural infarction showed a minimal elevation of serum LDH—306 and 456 μmole/min./L. (N = 206 ± 52). Most of the values for these enzymes in the control group were in the normal range. Exceptions were a serum LDH elevation five times higher than normal in a patient with left ventricular hypertrophy and ischemia, and serum GOT and GPT elevation in 2 patients—1 with left ventricular hypertrophy and the other with anterior wall ischemia.

![Fig. 1 (left). Curves showing linearity of LDH activity with sample size in serum and urine.](image1)

![Fig. 2 (right). Curve showing dialysis effect on urinary LDH activity in terms of nmole/min. of assay/kg. of body weight/hr.](image2)
In these patients GOT was elevated in the first week, but the increase in serum GPT lasted 4 weeks.

Urinary excretion of GOT was variable; it could be detected in only 20% of the urine samples assayed. Urinary GOT activity was most frequently found in samples collected in the first week after the infarction and only in occasional samples thereafter. In some individuals, urinary GOT could not be detected at any time. Individual urine samples showed activity as high as 25 nmole/min./kg./hr. (N < 5). Other than the rather regular presence in the urine during the first post-infarction week, no pattern for the occurrence of urinary GOT could be established. Urinary GPT was detected in less than 2% of the samples; it was never present in above-normal levels.

Urinary LDH was elevated significantly in 6 of the 11 patients with transmural infarction (Fig. 4). The day-to-day fluctuation of urinary LDH was considerable. The maximum levels occurred at variable times up to 2 weeks after the infarction. The mean urinary LDH excretion of the 11 patients with transmural infarction was elevated for about 10 days (Fig. 5) and was normal by the end of the experimental period. The control group (Fig. 5) and the 2 patients with nontransmural infarction had essentially normal LDH excretions. The high value in the control group on the first day was due to a high urinary LDH excretion on this day of only 1 patient whose excretory level dropped thereafter.

The normal ratio of LDH:HBD serum activity ranged from 1.8 to 1.9 in 10 of the patients with transmural infarction, the ratio decreased. Values as low as 1.3 were found, but ratios ranging from 1.6 to 1.7
were more common. There was no correlation between the elevation of enzyme activity and the ratio value. Individual samples from the control group had low ratios, but they were less common than in the transmural infarction group. In urine the ratio varied between 1.14 and 3.67; the value of the ratio appeared to be more characteristic of the individual patient than of the state of the disease.

**Acid Phosphatase**

In 5 of the 11 cases of transmural infarction, an increase in serum acid phosphatase was noted. The mean values for all groups are shown in Fig. 6. There was also an elevation in the control group and in the 2 patients with nontransmural infarction, though not so pronounced. After about 2 weeks, all groups showed an equally elevated level of serum acid phosphatase. The urinary excretion of acid phosphatase was quite variable, but generally within normal limits. An occasional specimen gave a low value.

The serum acid phosphatase was inhibited by copper about 50% and by tartrate about 15%. In urine, copper inhibited the acid phosphatase about 15% and tartrate 70%. These values are within the normal range, and there were no differences among the 3 groups of patients. Occasionally tartrate enhanced the serum acid phosphatase activity in patients with transmural infarction; we have never noted this before and have no explanation for it.

![Fig. 4. Curves showing urinary LDH excretion patterns in 11 patients with acute myocardial infarction. Double-headed arrows represent mean ± 2 S.D. (6.49 ± 4.36 n mole/min./kg./hr.).](image-url)
Fig. 5. Curves showing average urinary LDH activity in patients with transmural myocardial infarction (TMI) compared with control group (CG). Horizontal lines represent 2 S.E.'s either side of normal mean. Patients with nontransmural infarction (NI) not included in graph since 1 patient in group had no urinary LDH.

Abbreviations, Fig. 6-9: see legend Fig. 5.

Fig. 6. Curves showing average serum acid phosphatase activity in 3 groups of patients studied.

Fig. 7. Curves showing average daily serum alkaline phosphatase activity in 3 groups of patients studied.

Fig. 8. Curves showing average daily urinary excretion of alkaline phosphatase in 3 groups of patients studied.

Fig. 9. Curves showing average percentage inhibition of urinary alkaline phosphatase by L-phenylalanine in 3 groups of patients studied.
Alkaline Phosphatase

As shown in Fig. 7, the mean serum alkaline phosphatase gradually rose during the 5-week experimental period in all 3 groups of patients. In the transmural infarction group, 7 of the 11 patients had elevations. A similar elevation was noted in the control group, and a lesser one in patients with nontransmural infarctions.

Urinary excretion of alkaline phosphatase increased in 10 of the 11 patients with transmural infarction. Excretion was highest 5–10 days after the infarction and was still elevated in the fifth week (Fig. 8). The control and nontransmural groups gave essentially normal values. The urinary clearance of alkaline phosphatase in normals and in the control group in this study was less than 2 ml/hr./1.73 m². Nine of the 11 patients in the transmural infarction group showed values above this, with individual values as high as 8 ml/hr./1.73 m².

Inhibition of serum alkaline phosphatase by L-phenylalanine was the same in all groups with a mean value below 25%. In urine, however, there was a marked rise in the inhibition value in all patients with transmural infarction. In some cases, inhibitions of over 70% were noted as contrasted with the normal 20–30%. This increase was noted as early as the fifth day and was maintained until the end of the experimental period. Fig. 9 gives the mean values for all groups.

Discussion

Despite the marked rise in serum GOT in the first few days after transmural infarction, we found no consistent corresponding rise in urinary GOT; but it was most frequently found in the urine in the first postinfarction week. Kalmansohn and Kalmansohn (3) noted a severalfold elevation of urinary GOT in some patients with acute myocardial infarction. Their observations were based on the activity of freshly voided urine, and they demonstrated some instability of the urinary enzyme. Our results are based on 24-hr. collections. We have tested urine specimens with GOT activity and found the losses in the first 24 hr. rather insignificant. GPT rarely occurred in the urine of the patients in this study.

The source of elevated urinary LDH seen in some of the patients with transmural infarction is unknown. Urinary LDH is known to increase in renal disease (11–15), but there was no evidence of renal impairment in our patients as measured by creatinine clearance or protein excretion. Pfeffer and Frommhold (16), however, showed abnormal ¹²⁵I-hippuran renograms in patients with acute infarction with abnormalities evident up to 3 weeks after the infarction. Elevations
similar to those found here have been reported by Gault and Steiner (17) to follow renal and pulmonary infarctions. Increased urinary LDH activity has been claimed to originate from bacteria (18) or blood cells (19) in the urine. We have not been able to make such direct correlations as the sediments were not significantly different in urine with normal or elevated LDH activity.

In agreement with Schoenfeld (20), we found an elevation of serum acid phosphatase activity in over half the patients with transmural infarction. However, the control and nontransmural groups also showed some elevation. The major part of serum acid phosphatase activity is derived from the platelets, presumably liberated during clotting (21). The patients in all the groups were taking anticoagulants, and it is conceivable that this resulted in greater labilization of the platelets with transfer of the acid phosphatase to the serum. Further information on this point could be obtained by a simultaneous study of serum and plasma acid phosphatase.

Although the serum alkaline phosphatase was elevated in the transmural infarction group, it was similarly elevated in the control group; thus, it is not possible to attribute significance to it. Gault and Steiner (17) found a similar rise in serum alkaline phosphatases in cases of renal and pulmonary infarction.

The increased urinary alkaline phosphatase noted in the transmural infarction group does not seem to have originated from the serum because the L-phenylalanine inhibition of the urinary enzyme was quite different. In the serum, inhibition was normal; but all of the transmural infarction cases showed a marked increase in the inhibition of the urinary enzyme. This increase has been said to be characteristic of intestinal alkaline phosphatase (22), but it is hard to see how this is related to the present findings. The leukocytosis associated with acute myocardial infarction (23) and the elevated alkaline phosphatase of these leukocytes (24) suggested a possible source. We have studied the L-phenylalanine inhibition of peripheral blood leukocytes from several cases of acute transmural infarction and of infarcted cardiac muscle, but the inhibition values have been in the normal range.

At present we are uncertain as to the source of the urinary alkaline phosphatase that is inhibited by L-phenylalanine. Recently Warnock (25) showed that tissues other than intestine and some serums contain alkaline phosphatase fractions that are inhibited by L-phenylalanine. His results were obtained by staining electrophoretically separated isoenzymes in the presence and absence of inhibitor. Our assay methods for total and L-phenylalanine-inhibited alkaline phosphatase cannot show changes in isoenzyme distribution. However, if the L-phenyl-
alanine-inhibited fraction is preferentially cleared by the kidney, an increase in this fraction can easily be demonstrated in the urine; by our method it would escape detection in the serum.

References

